Inducing ovulation with hCG in a five-day progesterone-based fixed-time AI protocol improves the fertility of anestrous dairy cows under heat stress conditions

Running head: Benefits of hCG under heat stress

I. Garcia-Ispierto[a,b], F. De Rensis[c], X. Casas[d], F. Caballero[d], B. Serrano-Pérez[a,b], F. López-Gatius[b,e,*]

[a]Department of Animal Science, University of Lleida, Lleida, Spain.
[b]Agrotecnio Centre, University of Lleida, Lleida, Spain.
[c]Department of Veterinary Medical Science, University of Parma, Parma, Italy.
[d]Divasa-Farmavic, S.A. (DFV®), Gurb-Vic, Barcelona, Spain
[e]Transfer in Bovine Reproduction SLu, Barbastro, Spain

*Corresponding author. Tel: +34 660196295; fax +34 973 238264. E-mail address: lopezgatiusf@gmail.com (F. López-Gatius)

Abstract

This study compares the fertility effects of inducing ovulation using human chorionic gonadotropin (hCG) versus gonadotropin releasing hormone (GnRH) at the end of a 5-day progesterone(P4)-based protocol for fixed-time artificial insemination (FTAI) in heat-stressed and non-heat stressed anestrous lactating dairy cows. Heat stress (HS) was defined as an environmental temperature-humidity index (THI) > 72. A P4 intravaginal device (CIDR) was fitted for five days and GnRH administered upon CIDR insertion and a double dose (24 h apart) of prostaglandin F2a upon CIDR removal. Cows then received either GnRH (GnRH group; n = 506), or hCG (hCG group; n =493) 36 h after CIDR removal and were inseminated 50-56 h after CIDR removal. Ovulation failure was investigated in a subset of 425 cows: 223 and 202 receiving GnRH and hCG, respectively. Based on odds ratios, the interaction between treatment and HS had a significant effect on the ovulation failure rate (P = 0.01). This meant that compared to the rates recorded in non-heat-stressed, GnRH-treated cows (13%), ovulation failure in heat-stressed GnRH treated cows (25.3%) was more likely by a factor of 2.3 (P = 0.04), in non-heat-stressed hCG-treated cows (2.3%) was less likely by a factor of 0.16 (P = 0.02) and was not significantly different in heat-stressed hCG-treated cows (7%). Interactions between treatment and HS and between treatment and parity had a significant effect on the pregnancy rate (P = 0.0001 and P = 0.001, respectively). The treatment-HS interaction determined that compared to the rates recorded in non-heat-stressed, GnRH-treated cows (30.5%), pregnancy in heat-stressed GnRH-treated cows (17.6%) was less likely by a factor of 0.48 than the remaining cows (P = 0.001), whereas because of the treatment-parity interaction, compared to the rates recorded in primiparous, GnRH-treated cows (31.4%), pregnancy in GnRH-treated multiparous cows (18.9%) was less likely to conceive by a factor of 0.51 than the remaining cows (P

= 0.002). No significant effects of treatment on the rates of pregnancy loss or twin pregnancy were identified by binary logistic regression. In conclusion, hCG treatment given at the end of a 5-day P4-based protocol for FTAI improved ovulation and pregnancy rates in anestrous cows under conditions of HS and also had a beneficial impact on the pregnancy rate in anestrous multiparous cows throughout the year.

Key words: Ovulation failure; Parity; Pregnancy loss; THI, Bovine

1. Introduction

Heat stress (HS) is a main cause in low fertility in high-producing dairy cows [1,2]. This effect is not only exclusive of tropical areas. In fact, fertility of the herds located in a mild climate is often impaired by a hot environment, only the duration of HS is less than a tropical area [1,3]. This declined fertility is multifactorial, including anomalous follicular/luteal dynamics as a result of endocrine imbalances. In essence, the incidence of anestrus or lack of cyclicity increases dramatically under HS conditions [2,4]. Anestrus is mainly characterized by a lack of ovarian progesterone production [5,6] so that progesterone (P4)-based protocols for synchronizing estrus or inducing ovulation seem to better resolve anestrus related to a hot environment than gonadotropin releasing hormone(GnRH)-based protocols [1,5,7]. However, the likelihood of ovulation failure may increase up to 10 times in anestrous cows submitted to different P4-based protocols for fixed-time AI (FTAI) under HS, compared with their cyclic partners [8]. Furthermore, the risk of ovulation failure increases greatly during the warm season of the year even in cows showing clear estrous signs, including the presence of an ovulatory follicle [9,10]. Inducing ovulation with hCG in protocols for FTAI can promote ovulation in anestrous dairy cows, overcoming the negative effects of HS on the process of ovulation [11]. In a recent study, hCG treatment used to induce ovulation at the end of a P-4-based protocol for FTAI improved follicular/luteal dynamics of both cyclic and non-cyclic dairy cows compared to GnRH treatment [12]. However, although the incidence of ovulation failure in a total of 104 cows receiving hCG (4.8%) was appreciably lower than the rate recorded in a total of 98 cows receiving GnRH (12.5%), this difference was not significant, probably due to the small size of the study population [12]. Therefore, the objective of this study was to compare the effects of inducing ovulation using hCG or GnRH at the end of a 5-day P4-based protocol for FTAI on the incidence of ovulation failure and fertility in anestrous lactating dairy cows under HS conditions. Possible effects of hCG treatment on pregnancy loss rates were also investigated.

2. Material and Methods

2.1. Cattle and herd management

This study was performed on two commercial Holstein-Friesian dairy herds separated by one km in northeastern Spain. During the study period (May 2016 to October 2017), the mean number of lactating cows in the herds was 285 (Herd 1) and 3250 (Herd 2), respectively. Since the conception and pregnancy loss rates derived from anestrous cows were not significantly different between herds, data were grouped as one single herd. The mean annual milk production was 12,450 kg per cow and mean annual culling rate was 30%. Cows were grouped according to age (primiparous plus secundiparous versus multiparous), milked three times daily and fed complete rations. All cows were artificially inseminated and the herds were subjected to a weekly reproductive health program, as described elsewhere [8,12]. Only healthy cows free of detectable reproductive disorders and free of clinical diseases during the study period (Days −7 to 28 of insemination) were included. Exclusion criteria were the following disorders: mastitis, lameness, digestive disorders and pathological abnormalities of the reproductive tract detectable by ultrasonography. Spontaneous estrus was detected by visual observation several times throughout the day (Herd 1) or by using pedometers (Herd 2). All animals were reared within the herds.

2.2. Treatment, insemination and pregnancy diagnosis

During the weekly reproductive visit, open cows more than 60 days in milk with no estrous signs for at least 21 days and with no luteal structures detectable by ultrasound were alternately assigned on a weekly rotational basis to the groups: GnRH (n = 506), or hCG (n = 493). Cows in the GnRH group were treated with a controlled intravaginal progesterone-releasing device (CIDR) (CIDR, containing 1.38 g of progesterone; Zoetis Spain SL, Alcobendas, Madrid, Spain) plus GnRH (100 µg im; Cystoreline, CEVA Salud Animal, Barcelona, Spain) upon CIDR insertion. The CIDR was left in place for 5 d, and these animals were also given PGF2α (25 mg dinoprost im; Enzaprost, CEVA Salud Animal, Barcelona, Spain) on CIDR removal. Twenty-four and 36 h later, the cows received a second PGF2α dose and a second GnRH dose, respectively, and were inseminated 50-56 h after CIDR removal. The hCG group was treated with the same P4-based protocol but substituting the second dose of GnRH with 3000 IU hCG i.m. (Veterin Corion 750 UI/ml, Divasa-Farmavic, Gurb-Vic, Barcelona, Spain). All cows included in the study received a FTAI on Thursday (Herd 1) or Friday (Herd 2).

All cows were inseminated by five technicians with frozen-thawed semen from 18 bulls 14e20 h after the second GnRH dose or after hCG. The physical properties of the largest ovulatory follicle, uterus and vaginal fluid were used as reference to confirm estrus at FTAI. A cow was classified as ready for service when the CL was either less than 10mm or non-detectable, the diameter of the largest follicle was greater than 12mm and the uterus was highly turgid and contractile to the touch [13]. If a cow returned to estrus, its status was confirmed by examination per rectum, and the animal was inseminated at this time and recorded as non-pregnant. In the remaining cows, pregnancy diagnosis was performed by ultrasound 28 d post-AI. Pregnancy was confirmed 56 d post-AI in pregnant cows. Pregnancy loss was recorded when the second pregnancy diagnosis proved negative. Cows diagnosed as not pregnant received further treatment, but the corresponding subsequent data were not included in this experiment. This meant that a cow was included only once in the study. All gynecological exams and pregnancy diagnoses were performed by the last author.

2.3. Data collection and statistical analysis

The absence or presence of one corpus luteum (CL) or more at least 10mmin diameter was assessed by ultrasonography 7-9 days after AI in a subset of 425 cows (cows included in the study from May to December 2016): 223 and 202 for the GnRH and hCG groups, respectively. Corpus luteum size was taken as the mean of two measurements approximating the greatest length and width. Pregnancy rate was defined as the percentage of cows that became pregnant at FTAI out of the total number of cows in the corresponding group. The maximum temperature-humidity index (THI) on the day of hCG or GnRH treatment (36 h after CIDR removal) was used to evaluate the effects of HS (THI values higher than 72 [1]) on subsequent reproductive performance. It should be noted that in our geographical region, a clear negative effect of HS on the reproductive performance of lactating dairy cows has been extensively described [4,8-10]

The following data were recorded for each animal: herd; parturition and treatment dates; parity (primiparous versus multiparous); treatment (GnRH or hCG); maximum THI at treatment (≤72 versus > 72); milk production at AI (low producers < 40 kg versus high producers ≥40 kg); days in milk at AI (≤90 d versus > 90 d); ovulation failure (absence of a CL 7 days after AI in the subset of 425 cows); sire; AI technician; pregnancy after FTAI; presence of twins after FTAI; and pregnancy loss

Overall reproductive performance for the two treatment groups was evaluated using the chi-square test. The effect of treatment group on ovulation failure, pregnancy, twin pregnancy and pregnancy loss rate were analyzed by logistic regression (logistic procedure of PASW Statistics for Windows Version 18.0, SPSS Inc., Chicago, IL, USA) adjusting for lactation, days in milk, milk production, heat stress, sire and technician. The estimates and Wald 95% limits were used to calculate odds ratios and 95% confidence intervals (CI). The explanatory variables and interaction were evaluated using the backward elimination procedure and variables that significantly affected pregnancy or ovulation rate remained in the model [14]. The level of significance was set at $P < 0.05$. Values are expressed as the mean ± standard deviation (S.D.). The factors entered in the model as independent dichotomous variables (where 1 denotes presence and 0 denotes absence) were parity (multiparous), HS at treatment (THI > 72), days in milk (>90 d) and milk production (≥40 kg). Treatment, AI technician and sire (class variables) were considered factors in the analyses. Possible interactions between treatment and the dichotomous variables heat stress and parity were also analyzed. For the dependent variables twin pregnancy and pregnancy loss, only pregnant cows were included in the analysis.

3. Results

Mean milk production at AI, days in milk at AI, number of lactations and number of inseminations were 38.7 ± 11.2 kg, 131.8 ± 79.2 days, 2.4 ± 1.5 lactations and 3.2 ± 2.5 inseminations, respectively (mean ± SD). All cows were considered to be ready for insemination at FTAI. Two hundred and ninety two (29.2%) of the 999 cows enrolled became pregnant following FTAI, 10.6% of them (31/292) undergoing pregnancy loss. No cows experiencing ovulation failure became pregnant. Twin pregnancy was recorded in 15.8% of the cows (46/292). In the 78 week study period, a maximum THI higher than 72 was registered in the inseminating days of 39 weeks (50%), whereas HS was recorded in 22 (64.7%) of the 34 weeks of the study period for the subset of cows in which should that be analyzed for the ovulation failure rate. Values of each independent variable for each treatment and the effects of the different treatments on each dependent variable are shown in Table 1. According to chi-square tests the ovulation failure rate was significantly lower and the pregnancy rate significantly higher for the hCG group ($P < 0.05$).

Based on odds ratios (Table 2), the interaction between treatment and HS had a significant effect on the ovulation failure rate ($P = 0.01$). This meant that compared to non-heat-stressed GnRH-treated cows as reference, ovulation failure was more likely in heat-stressed GnRH-treated cows by a factor of 2.3 (P = 0.04), was less likely in non-heat-stressed hCG cows by a factor of 0.16 (P = 0.02) and was similar in heat-stressed hCG cows.

Based on odds ratios (Table 3), the interaction between treatment and HS and between treatment and parity has significant effects (two interactions) on the pregnancy rate (P = 0.0001 and P = 0.001, respectively). The treatment-HS interaction determined that heat-stressed, GnRH-treated cows were less likely to conceive by a factor 0.48 than the remaining of cows (P = 0.001), whereas the treatment-parity interaction meant that GnRH multiparous cows were less likely to conceive by a factor 0.51 than the remaining cows (P = 0.002).

No significant effects of the variables examined on rates of pregnancy loss or twin pregnancy were identified by binary logistic regression.

4. Discussion

This study sought to examine rates of ovulation failure, fertility and subsequent pregnancy loss in response to ovulation induction using hCG at the end of a 5-day P4-based protocol for FTAI in anestrous lactating cows under HS conditions. The points to be highlighted are that HS conditions, defined as an environmental THI >72 [1], had a negative effect on reproductive performance; hCG treatment led to a lower incidence of ovulation failure through both the warm and cool periods of the year and to a higher pregnancy rate under HS conditions compared to GnRH treatment; and multiparous cows were more likely to become pregnant after receiving hCG than GnRH. Thus, hCG treatment used to induce ovulation at the end of a short protocol for FTAI improves ovulation and pregnancy rates compared to GnRH treatment in anestrous lactating dairy cows under HS conditions. The study population was comprised of cows that had shown no estrous signs for at least 21 days and with no luteal structures at the start of synchronization. The absence of luteal structures in these cows suggests that most were anestrous cows. Although the addition of hCG to P4-based protocols has been noted to promote ovulation in anestrous dairy cows [11], anestrous cows are less sensitive to any kind of treatment [8,12]. Hence, a strong positive effect of hCG was recorded in heat-stressed cows. These results are consistent with previous findings derived from cyclic and noncyclic cows in which hCG lowered (although not significantly) the ovulation failure rate [12]. They also reinforce the results of a further study on cyclic cows in which reproductive performance was improved during the warm period of the year [15]. The latter study was performed in Northern Italy, a Mediterranean area which lies at a similar latitude and has similar climate conditions to the present study area. Finally, regardless of the cool or warm conditions, hCG treatment was related to an increased pregnancy rate in multiparous cows. Probably this is why the likelihood of ovulation failure for non-heat-stressed cows (THI $\leq$ 72 at AI) in the hCG group was lower (by a factor of 0.16) than that of their peers in the GnRH group. The reproductive age of the cow, measured usually as the number of calvings (lactation number), has been extensively related to decreased fertility in our geographical area [8,16,17] and elsewhere [18e20]. Given the positive effect of hCG treatment shown here on the fertility of multiparous anestrous cows irrespective of heat stress, a direct beneficial effect on the fertility of cyclic cows might be expected of this treatment in large field-scale trials.

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Table 1. Independent variables for each treatment and effects of the different classes on each dependent variable (n = 999).

Number of cows with	GnRH (n = 506)	hCG (n = 493)
Independent variables		
Parity (multiparous)	318 (62.8%)	332 (67.3%)
Milk production (≥ 40 kg)	249 (49.2%)	273 (55.4%)
Days in milk (> 90 d)	286 (55.9%)	300 (60.9%)
Heat stress (max THI > 72)	273 (54.0%)	234 (47.5%)
Dependent variable[a]		
Ovulation failure[b]	48/223 (21.5%)[a]	10/202 (5%)[b]
Conception rate	119/506 (23.5%)[a]	173/493 (35.1%)[b]
Pregnancy loss[c]	19/119 (16%)	12/173 (6.9%)
Twin pregnancy rate[c]	20/119 (16.8%)	26/173 (15%)

Treatments (all cows were FTAI 50-56 h after CIDR removal):

GnRH: cows given a dose of GnRH 36 h after CIDR removal.

HCG: cows given 3000 IU hCG 36 h after CIDR removal.

[a]Values with different superscripts differ within rows detected by the chi-square test ($P < 0.0001$).

[b] On a subset of 425 cows.

[c] Percentages in pregnant cows

Table 2. Odds ratios of the ovulation failure variables included in the final logistic regression model (n = 425).

Factor	Class	n	% ovulation failure	Odds ratio	95% confidence interval	P
Treatment x THI[a] interaction						0.01
	0 (≤ 72)	9/69	13.0	Reference		
	0 (> 72)	39/154	25.3	2.3	1.0-5.0	0.04
	1 (≤ 72)	2/87	2.3	0.16	0.03-0.75	0.02
	1 (> 72)	8/115	7.0	0.50	0.18-1.3	0.17

Hosmer and Lemeshow Goodness-of-fit test = 22.3; 2 df, P = 0.95.

R^2 Nagelkerke = 0.14.

Treatments (all cows were FTAI 50-56 h after CIDR removal):

GnRH: cows given a dose of GnRH 36 h after CIDR removal.

HCG: cows given 3000 IU hCG 36 h after CIDR removal.

[a]Treatment: 0, GnRH; 1, hCG. THI: maximum temperature-humidity index at treatment (between parentheses).

Table 3. Odds ratios of the pregnancy rate variables included in the final logistic regression model (n = 999).

Factor	Class	n	% pregnancy rate	Odds ratio	95% confidence interval	P
Treatment x THI[a] interaction						0.0001
	0 (≤ 72)	71/233	30.5	Reference		
	0 (> 72)	48/273	17.6	0.48	0.31-0.78	0.001
	1 (≤ 72)	90/259	34.7	1.1	0.66-1.8	0.89
	1 (> 72)	83/234	35.5	1.2	0.7-1.7	0.80
Treatment x parity interaction						0.001
	0-primiparous	59/188	31.4	Reference		
	0-multiparous	60/318	18.9	0.51	0.3-0.7	0.002
	1-primiparous	52/161	32.3	0.76	0.4-1.3	0.32
	1-multiparous	121/332	36.4	0.90	0.5-1.4	0.67

Hosmer and Lemeshow Goodness-of-fit test = 30; 2 df, P = 0.93.

R^2 Nagelkerke = 0.13.

Treatments (all cows were FTAI 50-56 h after CIDR removal).

GnRH: cows given a dose of GnRH 36 h after CIDR removal.

HCG: cows given hCG 36 h after CIDR removal.

[a]Treatment: 0, GnRH; 1, hCG. THI: maximum temperature-humidity index at treatment (between parentheses).